IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	Group Art Unit: 1653
BRODSKY	Examiner: Desai, Anand U.
Serial No.: 10/803,541	Confirmation No.: 6260
Filed: March 17, 2004	
Atty. File No.: 2848-53	DECLARATION OF GARY BRODSKY (under 37 CFR 1.132)
For: "PRODUCT AND METHODS FOR DIAGNOSIS AND THERAPY FOR CARDIAC AND SKELETAL MUSCLE DISORDERS"	*VIA ELECTRONIC FILING*

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, Gary Brodsky, declare as follows:

1. I am the inventor of the above-referenced patent application and am familiar with the application. I am a skilled artisan in the fields of molecular and cellular biology and have designed and performed or supervised the experiments described in the paragraphs below.

2. This Declaration is being submitted in conjunction with a Response to an Office Action having a mailing date of May 3, 2006.

3. The following discussion in paragraphs 4-5 is provided in response to the Examiner's rejection of Claims 1-8, 14-16, 38-40, 46-52 and 55-57 under 35 U.S.C. § 112, first paragraph. Specifically, the data presented in the following paragraphs demonstrate that my laboratory has shown that two different prelamin A prepeptides that differ by almost 47% sequence identity each promote myoblast fusion, myocyte activation, myocyte differentiation, and myocyte organization in the myoblasts. The peptides tested were the human (SEQ ID NO:2) and chicken (SEQ ID NO:17) prelamin A prepeptides, and the experiments described below were performed on mouse cells, further illustrating the ability of the invention to operate across species and with a variety of peptide variants within the scope of the present claims. In addition, I describe a further experiment using the human prelamin A prepeptide (SEQ ID NO:2) on rat

cells. As compared to human prelamin A prepeptide, the chicken peptide contains seven substitutions and two insertions. Accordingly, the chicken peptide is only about *53%* identical to the human peptide over the full length of the human peptide, and yet shares the same biological activity as the human peptide. The substitutions are at positions 1, 4, 5, 6, 9, 11 and 14, with respect to the human peptide, and the two insertions are between residues 12 and 13 with respect to the human peptide. In the above-identified application, I clearly teach at pages 35-37 that each of positions 1, 5, 6, 9, 11 and 14 can be modified without impacting activity of the peptide, and these experiments demonstrate that this teaching is correct. Moreover, I note that even a substitution at position 4, which is highly conserved among other animal species (see Fig. 2 of the application) results in a peptide that retains the biological activity of SEQ ID NO:2. Moreover, a double amino acid insertion (noting the chicken peptide has two additional amino acids as compared to human) also does not change activity. The data below clearly illustrate that the claimed peptides with up to 70% identity to SEQ ID NO:2 can readily be produced that have the biological activity of SEQ ID NO:2, as taught in the specification.

4. This example shows that the farnesylated carboxymethylated prelamin A pre peptide is the signal for skeletal muscle cell and cardiac cell differentiation, growth and repair.

The farnesylated and carboxymethylated human prelamin A pre peptide (SEQ ID NO:2) was chemically synthesized, and mitotically dividing C2C12 myoblasts were treated with the peptide at 1, 10 and 25 μg/ml in complete growth media containing 10% FBS. Six hours after peptide treatment at 1 μg/ml, cells have extended cytoplasmic processes and are migrating (Fig A). The muscle-specific cytoskeletal IF protein desmin can be seen localizing to the interface with the nuclear lamina and forming a filamentous skeleton at the locations where cells interact. In some areas, cell fusion can be seen after just 6 hours of peptide treatment. At 48 hours, depending on cell density, the cells have fused to form either sheets or myotubes (Fig. A). The non-directional nature in which the cells fuse suggests that, like a-factor, under normal conditions the peptide induces linear myotube formation by creating a signal gradient. The peptide also induces the elongated and kidney-shaped polarization of nuclei, with desmin aggregating at the central area.

To prove the peptide is a true myoblast differentiation signal and that it does not stimulate a general cellular fusion process, H9c2 rat cardiac myoblasts were treated with the peptide. These cells were previously considered to be of cardiac lineage in part because they

express heart-specific L-type calcium channels after prolonged exposure to retinoic acid during serum starvation induced differentiation. Differentiation of H9c2 cells in culture has previously led to fusion and multinucleate myotube formation closely resembling skeletal myoblast differentiation and sharing none of the physical characteristics of true cardiac myocytes (*i.e.*, resembling skeletal myotubes rather than cardiac myocytes). However, when exposed to the prelamin A pre peptide in growth media containing 10% FBS, H9c2 cells secrete an extensive desmin extracellular matrix within just six hours (Fig. B). After 48 hours, the treated cardiac myoblasts have formed a tissue sheet of mono- and binucleated cells interconnected by an extensive desmin extracellular matrix (Fig. B). Higher order intra- and intercellular organization is clearly evident in visible striations in the desmin staining pattern. Peptide-treated H9c2 cells show modest increases in lamin A/C and prelamin A expression (Fig. B) consistent with results indicating that the processing of preexisting prelamin A and lamin A pools is necessary for myoblast differentiation. Together, these results demonstrate that the covalently modified C-terminal prelamin A peptide signals cardiac and skeletal myoblast differentiation.

5. In this experiment, chemically synthesized and covalently modified prelamin A prepeptide from chicken (*i.e.*, SEQ ID NO:17 in the present application) was dissolved at 10 mg/ml in 50% DMSO/50% water. Actively proliferating C2C12 cells were treated with the chicken prelamin A peptide at 40 µg/ml on plastic chamber slides in complete DMEM media containing 10% fetal bovine serum (FBS). After 20 hours the cells were fixed in methanol/acetone and then immunostained with a rabbit anti-desmin primary antibody and a Texas Red conjugated anti-rabbit secondary antibody. Cover slips were mounted with media containing DAPI.

Within 20 hours, the chicken prelamin A peptide induced cell cycle arrest, myoblast morphogenesis and fusion. In the attached Fig. C containing the 10x images, the effect on cell density is clearly evident. Furthermore, the first panel has arrowheads pointing to some of the intensely staining round cells that are in the process of cell division. Two pools of separating DNA can be seen in these cells by increasing the magnification in PowerPoint to 300 or 400%, and in the 40x figure. In contrast, only one dividing cell can be identified (arrowhead) in the two fields of treated cells shown, directly demonstrating the chicken peptide has induced cell cycle arrest.

Peptide treatment results in a more distinct cytoskeletal staining pattern than the diffuse signal seen in the controls. Furthermore, large areas of fused cells are clearly evident throughout the fields, some of which are indicated by arrows.

In the attached Fig. D containing the 40x images, the actively dividing cells in the untreated control are again indicated by arrowheads, and the two hemispheres of dividing DNA are clearly visible. Even in this small field, numerous dividing cells are present in the control, as well as a number of cells which have just divided. At higher magnification, one can see that the desmin staining pattern in the control cells remains diffuse with little filamentous character (white arrow) as would be necessary for the cells to continue dividing. In the fields of peptide-treated cells a distinct and extensive filamentous desmin cytoskeleton is clearly evident as the cells have differentiated. The yellow arrow points to a cell cytoskeleton initiating at the nuclear interface and growing outward towards the plasma membrane, whereas in most treated cells cytoskeleton formation is already extensive at the 20 hour time-point. Differentiation is also indicated by the larger size and expanded DNA of the treated cells. Areas containing fused cells are also present in the 40x images.

These results demonstrate that the chicken prelamin A peptide induces cell cycle arrest, cellular morphogensis and cytoskeleton formation, and skeletal myoblast fusion when topically applied to proliferating myoblasts in complete growth media containing 10% FBS. Together with paragraph 4 above, these data demonstrate that the chicken prelamin A peptide functions analogously to the human prelamin A peptide as an extracellular signal for myoblast differentiation.

6. Finally, from the experiments described in paragraph 4 above, immunostaining of prelamin A (SEQ ID NO:4) in peptide-treated C2C12 myoblasts confirmed that prelamin A is involved in organizing chromatin, and forms a cytoskeleton and extracellular matrix during myoblast differentiation (data not shown). Internuclear connections containing prelamin A and desmin were seen forming that are the likely route by which the GFP-prelamin A fusion proteins and other proteins are transferred between nuclear domains during myotube formation. The intranuclear prelamin A signal became indistinguishable as the nucleus became encapsulated in prelamin A and desmin, indicating that prelamin A processing is responsible for protein redistribution. Prelamin A and desmin were intimately associated with and appeared to organize expanded heterochromatin during myoblast differentiation. Prelamin A and desmin enveloped

the differentiating nuclei and formed a distinct cytoskeleton concomitant with the disappearance of the intranuclear prelamin A signal. This was also seen in the masking of the DAPI signal. An extensive system of vertical prelamin A and desmin "pillars" was seen at the periphery of the nucleus. These experiments further illustrated the role of prelamin A and prelamin A processing in myoblast differentiation.

7. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.

Date: 11/3/06

By: _____
Gary Brodsky

FIG. A

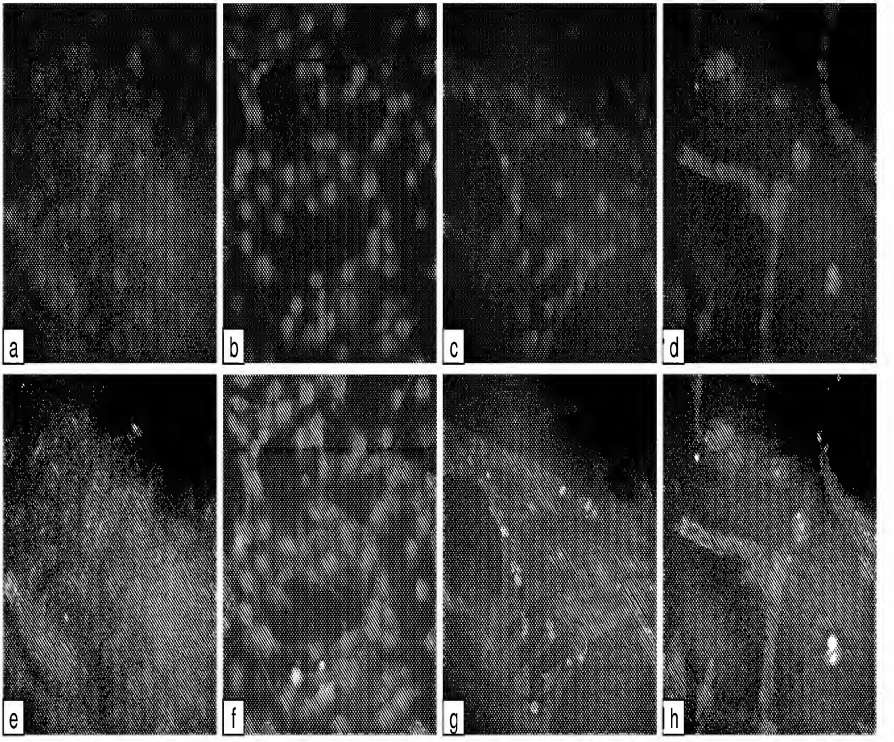

FIG. B

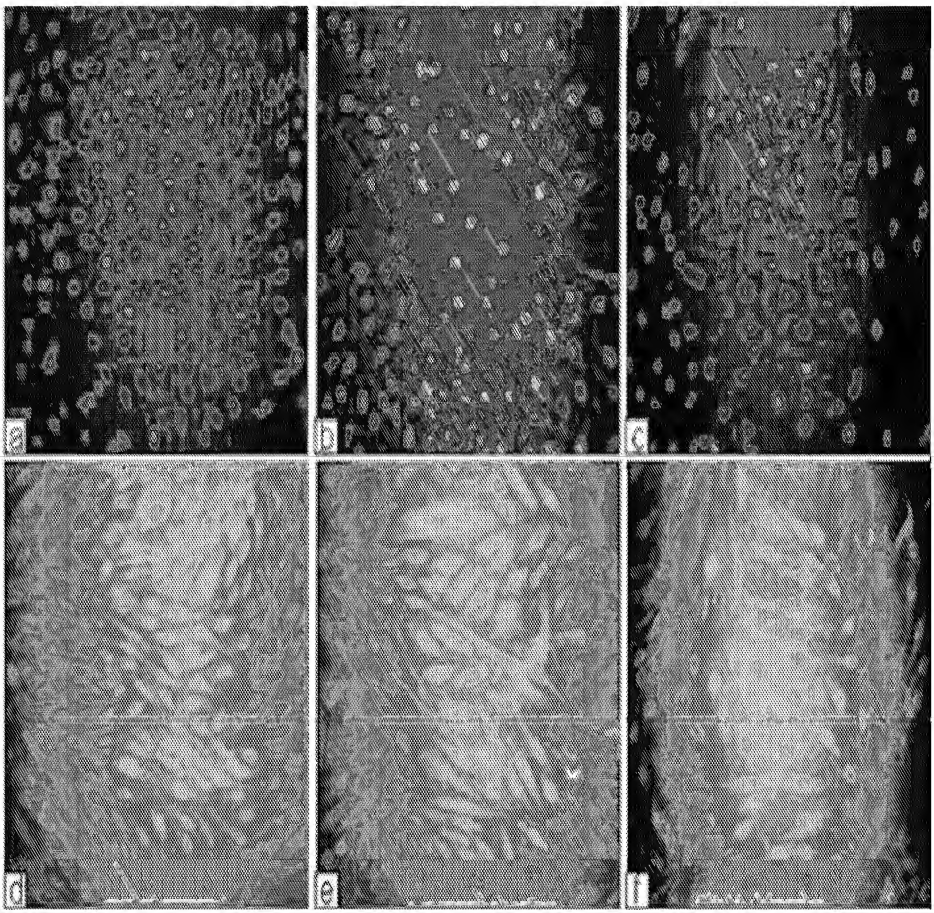

FIG. A

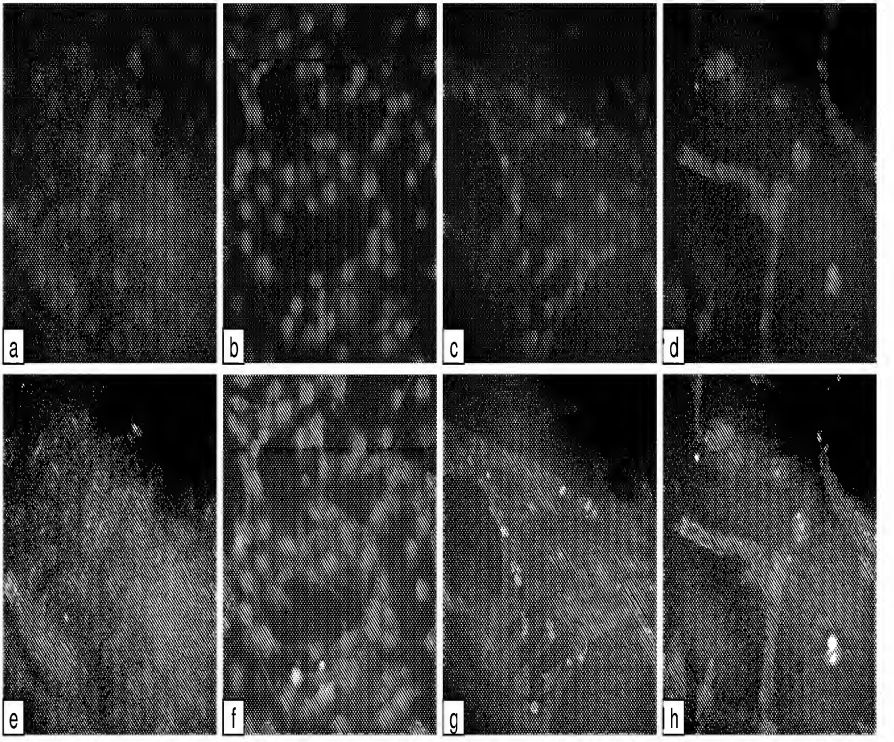

FIG. B

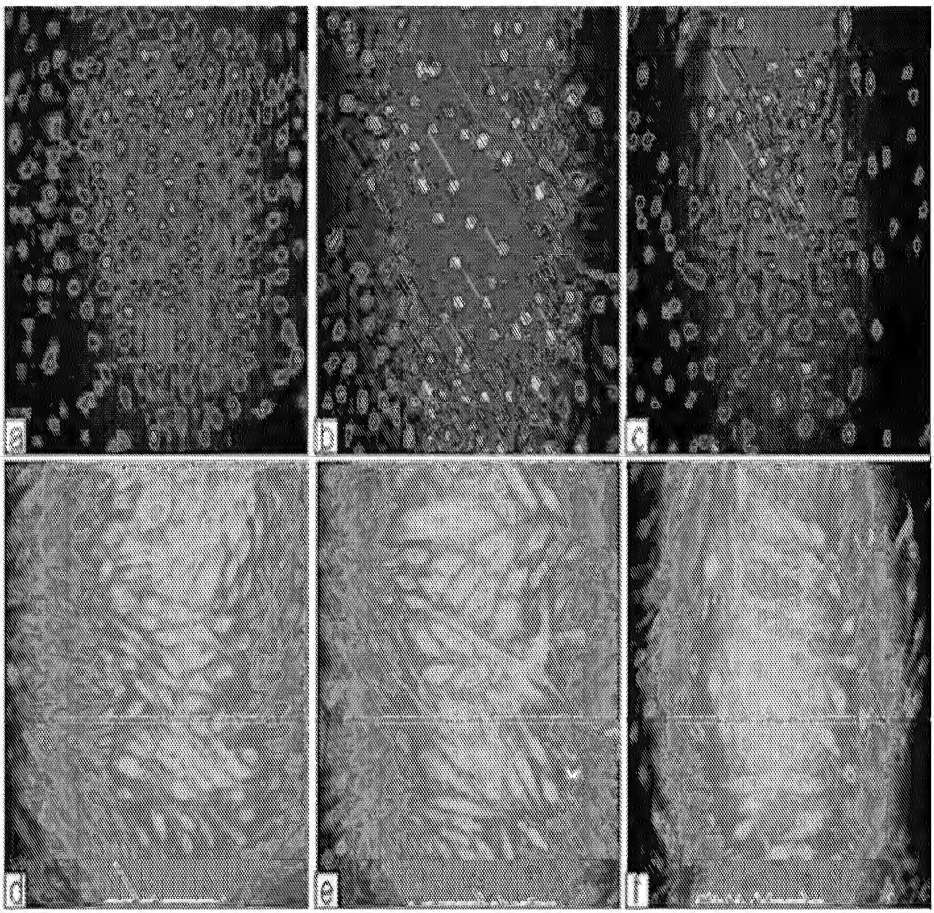

FIG. A

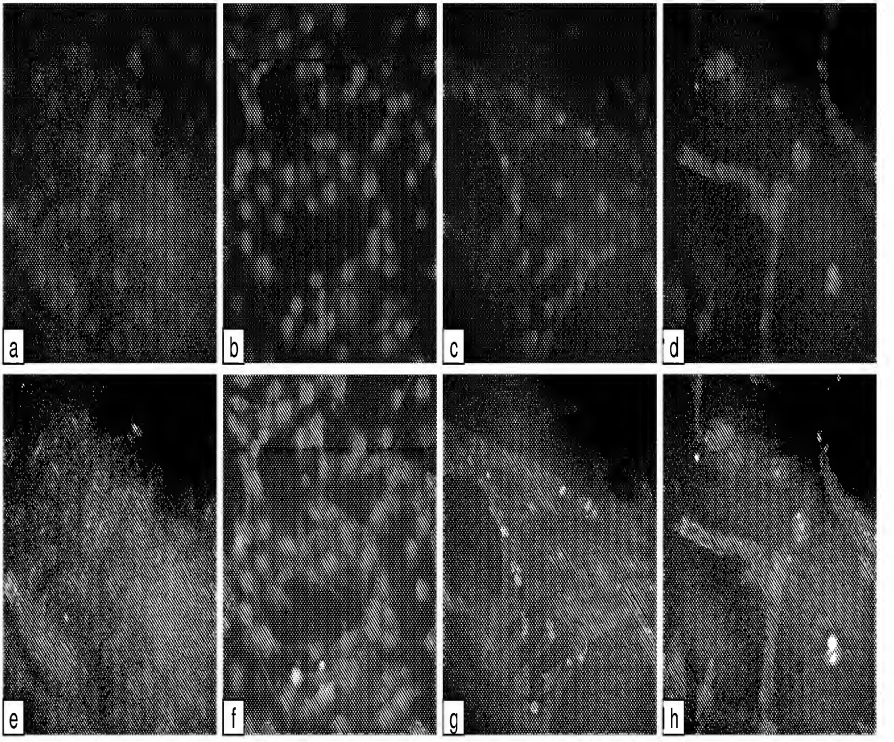

FIG. B

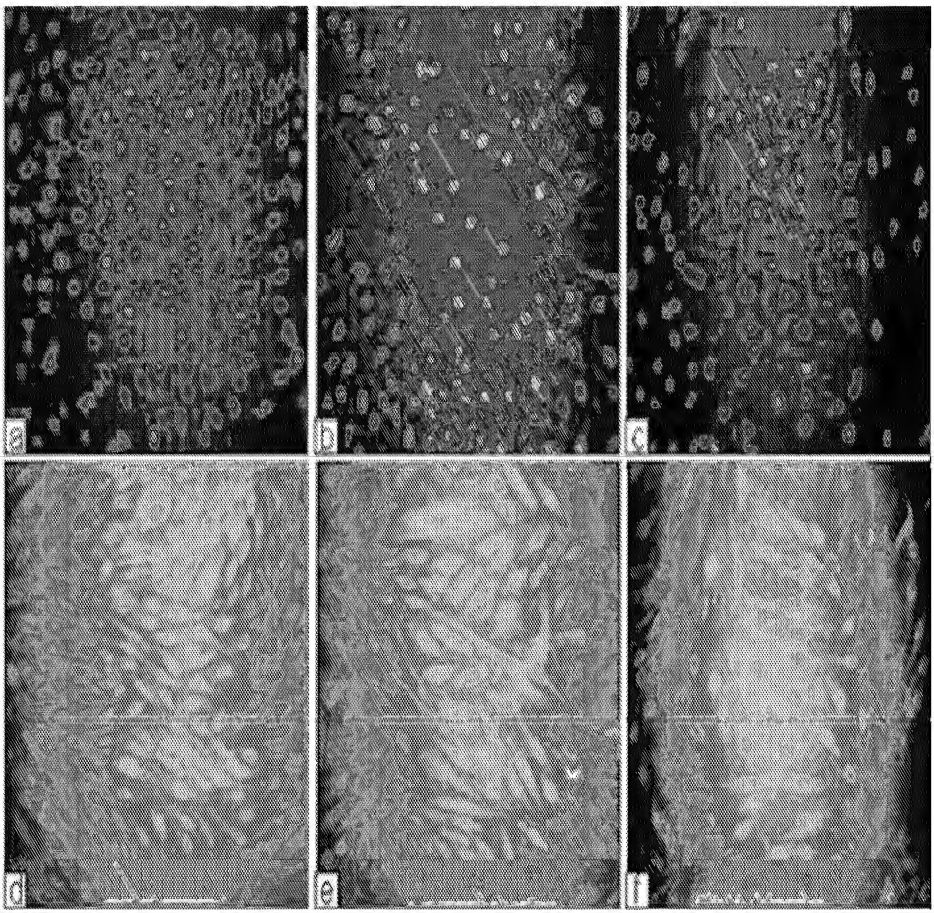

FIG. C

C2C12 20 hrs 10x magnification

red=desmin blue=DAPI

Chicken prelamin A peptide

Control | 40 ug/ml | 40 ug/ml

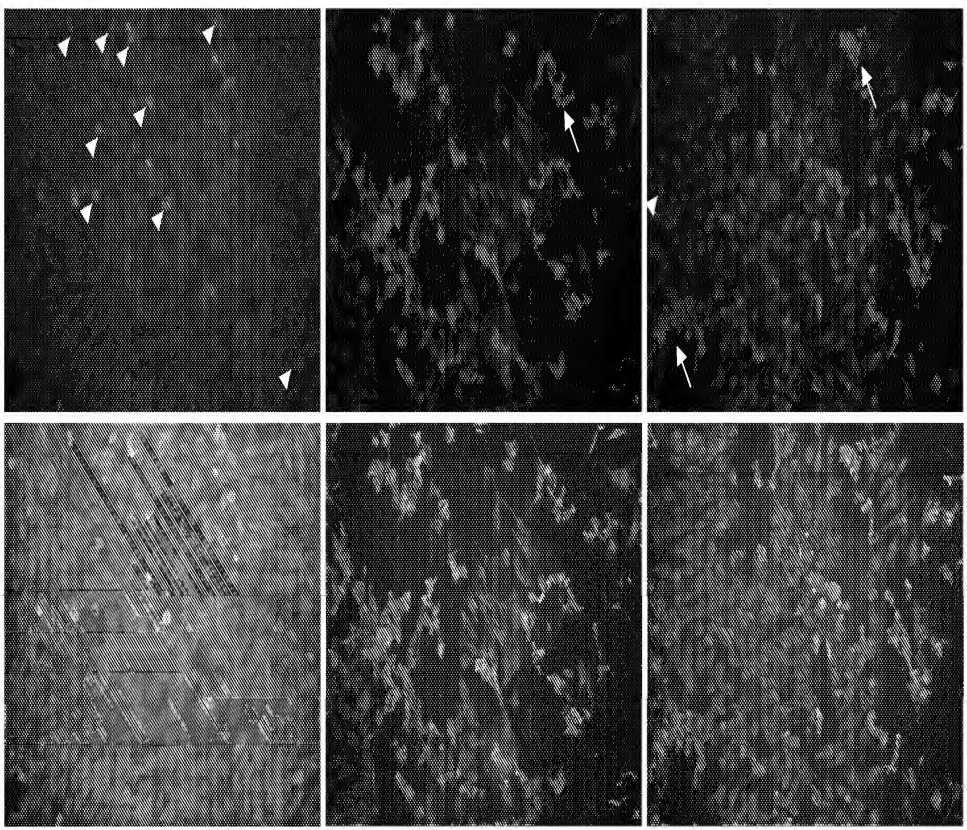

FIG. D

C2C12 20 hrs 40x magnification

red=desmin blue=DAPI

Chicken prelamin A peptide

Control | 40 ug/ml | 40 ug/ml